

From: <laura.n.winks@exxonmobil.com>
To: <coshita@oehha.ca.gov>
Date: 5/5/2009 2:51 PM
Subject: ExxonMobil Chemical submission to OEHHA: Prioritization
Comments Part II
Attachments: ExxonMobil DINP submission Part II.pdf; AttARoberts.PDF;
AttBKlaunig.PDF; AttCWilliams.PDF; AttDIrons.PDF; AttESwenberg.PDF

Dear Ms. Oshita:

This is the second of three emails containing comments from ExxonMobil Chemical regarding the prioritization of chemicals to be considered at the May 29 meeting of the Carcinogen Identification Committee. These comments pertain specifically to diisononyl phthalate (DINP).

The content of the three emails is as follows:

1st email: The email has attached Part I of ExxonMobil's comments, which is a summary of the pertinent data.

2nd email: This second email has attached Part II of our comments, which provides greater detail on the scientific data, and the following attachments:

- A Statement of Ruth Angela Roberts, Ph.D.
- B Statement of James Klaunig, Ph.D.
- C Statement of Gary Williams, M.D. and Michael Iatropoulos, M.D., Ph.D.
- D Statement of Richard Irons, Ph.D.
- E Statement of James Swenberg, Ph.D.

3rd email: The third email has attached a copy of Klaunig et al. (2003), which is a review of the peroxisome proliferation mechanism, based on data for DINP and other chemicals. Other expert body reviews of the DINP data (CPSC CHAP, EU Risk Assessment, NTP CERHR) have been provided to the CIC by OEHHA and/or are readily available on the Internet. Because Klaunig et al. (2003) is not readily available on the Internet and is not cited by OEHHA, we are providing a copy. It is 6 MB -- if not received please let us know and we will break it down.

ExxonMobil would be pleased upon request to provide a copy of any other study cited in our comments.

If the CIC or OEHHA have any questions or need additional information, please contact me or any of the following scientists:

Ammie Bachman, Ph.D., ammie.n.bachman@exxonmobil.com, (908) 730-2082
Bob Barter, Ph.D., robert.a.barter@exxonmobil.com, (908) 730-2153
Michael Bird, Ph.D., michael.g.bird@exxonmobil.com, (908) 730-1060
Rick McKee, Ph.D., DABT, richard.h.mckee@exxonmobil.com, (908) 730-1037

Thank you.

(See attached file: ExxonMobil DINP submission Part II.pdf)

(See attached file: AttARoberts.PDF)(See attached file: AttBKlaunig.PDF)
(See attached file: AttCWilliams.PDF)(See attached file:
AttDIrons.PDF)(See attached file: AttESwenberg.PDF)

Regards,
Laura N. Winks
Oxo Americas Regulatory Affairs Advisor
ExxonMobil Chemical Company
Bus Phone: 281-870-6439
laura.n.winks@exxonmobil.com

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COMMENTS ON PRIORITIZATION OF DIISONONYL PHTHALATE (DINP)
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PART TWO
IN-DEPTH DISCUSSION OF PERTINENT DINP DATA

ExxonMobil Chemical is submitting these comments in response to notification by the California Office of Environmental Health Hazard Assessment (OEHHA) of 38 chemicals for consultation by the Carcinogen Identification Committee (CIC) at its meeting on May 29, 2009. The purpose of these comments is to provide the CIC with relevant scientific information to support the CIC's evaluation of diisononyl phthalate (DINP), and specifically the CIC's recommendation to OEHHA concerning the level of priority that should be assigned to DINP for future consideration as a candidate chemical for listing under Proposition 65. Part One of the comments presented a summary of the strong body of evidence that neoplasms observed in rodent studies of DINP are caused by mechanisms that are not relevant for humans. This Part Two of the comments provides greater detail on that body of evidence.¹

Section I discusses the human data available for DINP. There are no epidemiology studies, but there are other data that demonstrate DINP does not affect humans in the same manner as rodents.

Section II summarizes the *in vivo* and *in vitro* mutagenicity and genotoxicity tests on DINP. These uniformly demonstrate that DINP is not a genotoxic substance.

Unlike most other substances, DINP has been studied in primates (including human cell cultures) as well as rodents. Section III summarizes the primate data which show no toxic effects even from very large doses of DINP.

Section IV discusses the conclusions of several expert body reviews of DINP in the past decade, which have found that the tumors observed in rodent studies are not relevant for human risk assessment.

Section V then examines each type of cancer lesion seen in rodents – liver tumors, mononuclear cell leukemia (MNCL), and kidney tumors – and explains why they are not relevant for human risk assessment.

The conclusion from this large body of evidence is that DINP is very unlikely to cause cancer in humans and therefore should not be a priority for evaluation and consideration of listing under Proposition 65.

¹ ExxonMobil recognizes that this Part Two addresses a large body of information. Questions or requests for additional information (including copies of studies cited herein) may be directed to any of the following individuals:

Ammie Bachman, Ph.D., ammie.n.bachman@exxonmobil.com, (908) 730-2082

Bob Barter, Ph.D., robert.a.barter@exxonmobil.com, (908) 730-2153

Michael Bird, Ph.D., michael.g.bird@exxonmobil.com, (908) 730-1060

Rick McKee, Ph.D., DABT, richard.h.mckee@exxonmobil.com, (908) 730-1037

Laura Winks, laura.n.winks@exxonmobil.com, (281) 870-6439

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I. AVAILABLE HUMAN DATA SUPPORT THE CONCLUSION THE DINP IS NOT LIKELY TO CAUSE CANCER IN HUMANS

There are no epidemiology studies on the carcinogenic potential of DINP. Nevertheless, there are some other types of human data which support a conclusion that tumors observed in rodent studies of DINP are unlikely to occur in humans. As discussed further in Section V.A.5 below, studies in human cell cultures (Baker *et al.*, 1996; Hasmall *et al.*, 1999; Kamendulis *et al.*, 2002) show a lack of the peroxisome proliferator response observed in rodents as a key event leading to development of liver tumors. In addition, because of differences in human and primate absorption of phthalates, internal doses equivalent to those required to produce tumors in rodents cannot be achieved in humans. Further, data on human exposure to DINP show that exposures are orders of magnitude below levels required to produce tumors in rodents (see Section V.A.6).

II. DINP IS NOT GENOTOXIC

A genotoxic chemical can damage cellular DNA and thereby trigger cancerous growth. DINP is not such a substance. As shown in Table 1, DINP has been evaluated in multiple *in vivo* and *in vitro* genotoxicity/mutagenicity assays and has been negative in all of them. Even at very high doses of DINP, the tests have found neither DNA mutations nor chromosomal damage.

In vivo, a micronucleus test in mouse bone marrow found no evidence of chromosomal damage following administration of 2 g/kg/day (2000 g/kg/day) of DINP for two consecutive days (McKee, *et al.*, 2000). In a rat bone marrow chromosome aberration test, DINP was negative at doses up to approximately 5.0 grams/kg/day for five days, for a cumulative dose of up to 25 g/kg (Microbiological Associates, 1981).

In vitro, DINP has been tested in the *Salmonella* mutagenicity assay and found to be without activity in plate incorporation assays sponsored by the NIEHS (Zeiger *et al.*, 1985) and in both plate incorporation and pre-incubation assays conducted by producing companies (McKee *et al.*, 2000). DINP also tested negative in the mouse lymphoma test and the Balb/3T3 cell transformation assay (Barber *et al.*, 2000), as well as the unscheduled DNA synthesis test in rat hepatocytes (Litton Bionetics, 1981). In an *in vitro* cytogenetics test in CHO cells, DINP was without activity even though the highest levels tested produced evidence of visible precipitation in the cell cultures (McKee *et al.*, 2000).

Thus, the conclusion that DINP is not mutagenic or genotoxic is supported by a strong database.

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Table 1.
 Summary of Genetic Toxicology Information on DINP

Test System	Result	Reference
Salmonella (plate incorporation)	negative (+/- S9)	McKee <i>et al.</i> , 2000
Salmonella (preincubation)	negative (+/- S9)	McKee <i>et al.</i> , 2000; Zeiger <i>et al.</i> , 1985
Mouse lymphoma	negative (+/- S9)	Barber <i>et al.</i> , 2000
Cytogenetics (<i>in vitro</i>)	negative (+/- S9)	McKee <i>et al.</i> , 2000
Unscheduled DNA synthesis (rat hepatocytes)	negative	Litton Bionetics, 1981
Mouse micronucleus test	negative	McKee <i>et al.</i> , 2000
Cytogenetics (rat bone marrow)	negative	Microbiological Associates, 1981
Transformation assay (Balb/3T3)	negative	Barber <i>et al.</i> , 2000

III. STUDIES IN PRIMATES SHOW NO EVIDENCE OF A POTENTIAL CANCER RESPONSE

Often, toxicological data on a chemical is limited to studies in rats and mice. For DINP, however, there is also an unusually large amount of data from studies in non-human primates – species that are much more closely related to humans than are rodents – as well as some *in vitro* data for humans. This primate data provides the best basis for determining whether chronic effects seen in rodents can reasonably be anticipated to occur in humans. Because monkeys are more closely related to humans than are rodents, primate studies provide a more relevant animal model for evaluating DINP than do rodent studies (*e.g.*, Mazue and Richez, 1982). This is supported not only by the taxonomic, evolutionary, and genetic evidence that places humans in the primate family, but also by toxicokinetic and mechanistic data.

There have been two *in vivo* studies of DINP in non-human primates. In one, cynomolgus monkeys were treated with DINP for 14 days at levels up to 500 mg/kg/day (Pugh *et al.*, 2000). In the other, marmosets were treated with levels up to the very high dose of 2500 mg/kg/day for 90 days (Hall *et al.*, 1999). (For a 70 kg human, this dose would be about six ounces per day.) In both of these primate studies, there was no evidence of treatment-related effects, including no changes in liver or kidney weights and no treatment-related changes in histopathology, even at the very high levels of treatment.

These studies were subchronic, versus the chronic bioassays in rodents. However, the lack of adverse effects in the primate studies even at very high doses is in contrast to the progression of pathology in rodents. For example, liver and kidney weights were increased in a 28-day study of rats (BIBRA, 1986). Liver weight increases were seen as early as 1 week after the beginning of treatment in the rat chronic bioassay (Moore, 1998a). Thus, the primate studies strongly indicate that primates are not adversely affected by DINP in the manner of rodents.

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As discussed in Section V.A below, liver tumors in rodents treated with DINP are due to the peroxisome proliferation mechanism. There was no evidence of peroxisome proliferation in either human hepatocytes (Baker *et al.*, 1996; Hasmall *et al.*, 1999; Kamendulis *et al.*, 2002) or other primate hepatocytes tested under *in vitro* conditions (Benford *et al.*, 1986; Kamendulis *et al.*, 2002). Thus studies from several laboratories using hepatocytes from different individuals or different species of primates have demonstrated that a peroxisome proliferator response is not elicited by DINP in humans and other primates. Further, as discussed in Section V.A.6, toxicokinetic data in primates and human volunteers show that the high internal doses associated with adverse effects in rodents cannot be achieved in humans.

Thus, primate studies and *in vitro* human and primate tests show no evidence of potential carcinogenicity, even under conditions that unquestionably would in rodents provoke responses that are part of the progression to cancer in those species.

IV. EXPERT BODY REVIEWS HAVE DETERMINED THAT DINP IS UNLIKELY TO POSE A HUMAN CANCER RISK

DINP has not been listed as a carcinogen, nor even considered for listing, by the International Agency for Research on Cancer (IARC) or the National Toxicology Program (NTP).² However, DINP has been the subject of other rigorous scientific reviews, which have concluded DINP is unlikely to pose a cancer risk to humans.

- A Chronic Hazard Advisory Panel (CHAP) of the Consumer Product Safety Commission (CPSC), consisting of seven independent experts, held three public meetings in the year 2000 to evaluate the toxicological data for DINP. The CHAP's report was published in 2001 (CPSC, 2001). The CHAP concluded that: the criteria for the alpha_{2u}-globulin mechanism were met and therefore the kidney tumors observed in male rats are rat-specific; the MNCL observed in Fisher 344 rats treated with DINP is of questionable significance due to its high and variable background and possible strain specificity; and the liver tumors in rodents are not relevant for human risk assessment because, even if DINP could activate the PPAR α mechanism in humans, the dose that would be required to do so is far in excess of any reasonably anticipated human exposures (CPSC, 2001).
- In 2003 a workgroup of the International Life Sciences Institute (ILSI) Risk Science Institute reviewed the relationship of peroxisome proliferation and liver tumors in rodents, publishing its results as Klaunig *et al.* (2003). This effort was to update the 1995 ILSI workshop on peroxisome proliferation and rodent tumors, reported by Cattley *et al.* (1998). DINP was one of the peroxisome proliferators used to develop the workgroup's conclusion that the rodent mode of action for liver tumors from such compounds is not relevant to humans.

² The Natural Resources Defense Council has nominated DINP for consideration by IARC (IARC, 2008), but to date IARC has not scheduled DINP for consideration (IARC, 2009).

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- The European Union (EU) has conducted a very thorough risk assessment of DINP, with input from governmental scientists throughout Europe (ECB 2003a; 2003b). The EU risk assessment concluded that the liver tumors observed in rodents are due to a peroxisome proliferation process that is not relevant to humans, the kidney tumors in male rats were due to an α_{2u} -globulin process that is not relevant to humans, and the MNCL was a strain-specific effect not relevant to humans (ECB, 2003a, Section 4.1.2.8). On the basis of its review, the EU has concluded that there is no basis to expect human risk of cancer, reproductive or developmental, or any other health effect from exposure to DINP. Accordingly, the EU also has determined that DINP should not be classified or labelled for human health effects, including no cancer designation (EC, 2000).

These consensus opinions support the conclusion that DINP is highly unlikely to cause adverse human health effects.

ExxonMobil notes that, while the U.S. Environmental Protection Agency (EPA) has reviewed the data for DINP, it *has not* made a final determination regarding the carcinogenicity of DINP. EPA undertook its review in response to a petition to list DINP under Section 313 of the Emergency Planning and Community Right-to-Know Act (EPCRA). OEHHA has provided to the CIC a 2000 Federal Register notice in which EPA proposed to list DINP in part based on the animal cancer data (Fed. Reg., 2000). However, after receipt of comments, EPA published a revised notice on June 14, 2005, in which it reserved judgment on the potential for DINP to cause cancer in humans (Fed. Reg., 2005). EPA accepted further comments and to date has not issued a final decision.

V. CANCER LESIONS OBSERVED IN RODENT BIOASSAYS OF DINP ARE NOT RELEVANT TO HUMANS

In rodents, DINP at high doses produces liver tumors in rats and mice, kidney tumors in male rats, and mononuclear cell leukemia (MNCL) in rats. However, there is a substantial body of research that provides compelling evidence that these tumors in rodents are not relevant for human health assessment. The overwhelming weight of the evidence is that DINP cannot reasonably be anticipated to cause cancer in humans. Numerous independent scientists agree with this assessment, based on application of generally accepted scientific principles.

A. Liver Tumors Observed In Rodents Are Due to Peroxisome Proliferation

Liver tumors have occurred in rats and mice exposed to high doses of DINP (Moore 1998a and 1998b).³ DINP is in a class of chemicals known as "peroxisome proliferators" – chemicals that induce an increase in the size and number of a subcellular organelle known as a "peroxisome" in the liver cells of rodents. Many peroxisome proliferators are known to cause liver tumors in rodents.

³ In various reviews of DINP, the Moore studies alternatively are referred to as the Aristech studies (Aristech Chemical Company sponsored the studies) and as the Covance studies (Covance Laboratories conducted the studies).

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Because many peroxisome proliferators are important pharmaceutical agents (the fibrate class of hypolipidemic drugs), the toxicology of these chemicals has been extensively studied; a substantial amount of such work also has been performed with DINP and the related phthalate compound, di(2-ethylhexyl) phthalate (DEHP). This has resulted in an extensive body of work that demonstrates that rodent liver tumors associated with peroxisome proliferation are not relevant for assessing potential human carcinogenicity. In fact, based on this evidence, the International Agency for Research on Cancer (IARC) and the International Life Sciences Institute (ILSI) have developed criteria for determining when tumors in rats and mice can be judged as not relevant to humans because they are secondary to peroxisome proliferation (IARC, 1995; Cattley *et al.*, 1998; Klaunig *et al.*, 2003).

1. Background on Peroxisome Proliferation

It has been known for some years that certain substances – including some phthalate esters – produce a specific set of changes characterized as “peroxisomal proliferation” in livers of rats and mice following treatment at high levels. It also has been known for some years that chronic dietary administration of DEHP can produce liver tumors in rats and mice (Kluwe, 1982). A link between peroxisome proliferation and hepatocarcinogenesis in rats and mice which was proposed 20 years ago (Reddy and Arzanoff, 1980) has engendered considerable research, because humans do not appear susceptible to peroxisomal proliferation. For example, clinical studies of humans exposed for long periods to hypolipidemic drugs that are strong rodent peroxisome proliferators and are rodent hepatocarcinogens (reviewed in Ashby *et al.*, 1994; Bentley *et al.*, 1993) have shown no indication of any increase in cancer associated with those substances. As a result of this research, there is now a large body of evidence that demonstrates that the mechanism by which nongenotoxic peroxisome proliferators such as DINP and DEHP cause liver carcinogenicity in rodents is not relevant for humans (Ashby *et al.*, 1994; Kluwe, 1994; Bentley *et al.*, 1993; Lake, 1995; Huber *et al.*, 1996; Williams and Perrone, 1997; Cattley, *et al.*, 1998; Klaunig *et al.*, 2003). Rats and mice are uniquely susceptible to the morphological, biochemical, and carcinogenic effects of peroxisome proliferators, while non-human primates and humans are completely non-responsive or refractory (*e.g.*, Bentley *et al.*, 1993; Elcombe *et al.*, 1996; Hall *et al.*, 1999; Huber *et al.*, 1996; Kurata *et al.*, 1998; Pugh *et al.*, 2000).

The research was substantially advanced by the work of Issemann and Green (1990) who showed that peroxisome proliferator activity is mediated through a specific receptor (the peroxisome proliferator-activated receptor α , or PPAR α), and by the demonstration that a mouse strain which lacks this receptor (PPAR α -null mice) does not express peroxisomal proliferation or develop liver tumors following treatment for 12 months with a strong peroxisome proliferating agent (Peters *et al.*, 1997).⁴ These studies demonstrated an absolute requirement for activation

⁴ Peters, *et al.* (1997) compared the response of PPAR α -deficient and normal PPAR α mice following long-term administration of a potent peroxisome proliferating agent. The PPAR α mice developed a 100% incidence of liver tumors following test material administration whereas the PPAR α -deficient animals failed to develop tumors and did not exhibit liver cell proliferation of any type or peroxisome proliferation.

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of the PPAR α receptor and expression of peroxisome proliferation in the development of rodent liver cancer.

There have been three particularly important reviews by independent scientific bodies of the evidence on peroxisome proliferation and its relationship to carcinogenic induction (IARC, 1995; Cattley *et al.*, 1998; Klaunig *et al.*, 2003). All three groups concluded that peroxisome proliferation-mediated rodent liver cancer has no practical significance to human health.

The first review was a 1994 working group of IARC to consider the relevance to humans of peroxisome proliferation as a generic mechanism (IARC, 1995). The IARC working group concluded that, when liver tumors in rats and mice were secondary to peroxisomal proliferation, this information could be used to modify the overall evaluation of the carcinogenicity data. One particular contribution by this group was to delineate the categories of evidence that could be used to establish whether rodent liver tumors are the consequence of a peroxisomal proliferation process.

The second review was by an international consensus workshop organized by the ILSI Health and Environmental Sciences Institute in December 1995, to consider specifically whether peroxisome proliferating compounds pose a liver cancer hazard to humans (Cattley *et al.*, 1998). The symposium included approximately 100 scientists from government agencies, academia and industry, including leading researchers in the field from the United States and Europe. The report of the workshop states, "The conclusion was reached that it is unlikely that peroxisome proliferators are carcinogenic to humans under anticipated conditions and levels of exposure, although their carcinogenic potential cannot be ruled out under extreme conditions of exposure." (Cattley *et al.*, 1998, p. 57). One particular contribution of the ILSI working group was to delineate the criteria that could be used to define a substance as a peroxisome proliferator.

In 2001, the ILSI Risk Science Institute (ILSI RSI) formed a workgroup to review the information that had become available since 1995 on the relationship of peroxisome proliferation and liver tumors in rodents. The results of a series of meetings of that workgroup are presented in a paper titled "PPAR α Agonist-Induced Rodent Tumors: Modes of Action and Human Relevance" (Klaunig *et al.*, 2003). DINP is one of the examples of a peroxisome proliferator discussed in the document. The workgroup concluded:

In summary, the weight of evidence overall currently suggests that the rodent [mode of action] for liver tumors is not likely to occur in humans, taking kinetic and dynamic factors into account. This conclusion is based upon evaluation of the existing body of evidence and would apply to the consequences of exposure to *known* examples of PPAR α agonists.

(Klaunig *et al.*, 2003, p. 693.) DINP is a known example of a PPAR α agonist that was part of the basis for the workshop conclusions. Therefore, the conclusion of the ILSI RSI workgroup is that the liver tumors that occur in rodents treated with DINP are not likely to occur in humans.

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Thus, there was a consensus in the scientific community that peroxisome proliferators presented, at the most, a theoretical risk that could be expressed only under the most extreme conditions of exposure. The critical questions to evaluate the DINP data then become: (1) Is DINP a peroxisome proliferator; *i.e.*, have the ILSI criteria been met? (2) Were the rodent liver tumors the consequence of a peroxisomal proliferation process, *i.e.*, have the IARC criteria been met? (3) Is there any possibility of cancer, even under extreme circumstances? and (4) If a theoretical possibility exists for human cancer, can the extreme exposure levels necessary be achieved? As shown below, the answers to these questions demonstrate that DINP cannot reasonably be anticipated to cause cancer in humans.

2. DINP Is a Peroxisome Proliferator Under the ILSI Criteria

As stated above, the 1995 ILSI workshop developed criteria for determining whether rodent liver tumors are the consequence of a peroxisomal proliferation process. Table 4 of Cattley *et al.* (1998) (reproduced here as Table 1) sets forth the minimum database to support characterization of a *non-genotoxic hepatocarcinogenic substance* as a peroxisome proliferator. DINP is a non-genotoxic substance as shown in Section II, above. DINP is a hepatocarcinogenic substance, as demonstrated by the observation of increased liver tumor incidence in rats and mice fed high doses of DINP (336 mg/kg/day in female mice; 700 to 900 mg/kg/day in male mice and in rats) (Moore 1998a; b). DINP also meets the criteria in Table 1, as shown in the text below.

Table 1.
 Minimum database to support characterization of a nongenotoxic hepatocarcinogenic substance as a peroxisome proliferator (from Table 4, Cattley *et al.*, 1998)

Key Element	Criteria	Measure
Gross hepatic morphology	Hepatomegaly	Increase in relative liver weight
Peroxisomes	Peroxisome proliferation	Increase in hepatocyte peroxisomes (V/V) by morphometry
Cell proliferation	Enhanced replicative DNA synthesis	Increase in hepatocellular BrdU nuclear labeling by light microscopy

- (1) Hepatomegaly: DINP treatment causes significant increases in liver weights in rats and mice as documented in BIBRA (1986), Barber *et al.* (1987), Lington *et al.* (1997), Moore (1998 a; b), Valles *et al.*, (2003), and Smith *et al.* (2000).
- (2) Peroxisome Proliferation: That DINP produces peroxisomal proliferation in rats was first documented by Barber *et al.* (1987) and in the original study report (BIBRA, 1986). These reports also documented an increase in peroxisomal enzymes, also shown in Moore (1998a; b), Valles *et al.* (2003), and Smith *et al.* (2000). A study in mice demonstrated the dose-response relationship of DINP treatment to peroxisome proliferation, utilizing light microscopy, morphometric evaluation and peroxisomal enzyme induction (Kaufmann *et al.*, 2002).

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- (3) Cell Proliferation: The induction of cell proliferation by DINP treatment in rat and mouse liver was first documented by Moore (1998a; b) and subsequently confirmed by Smith *et al.* (2000) and Valles *et al.* (2003). The enhanced cell proliferation was observed in the same hepatic compartment (perivenous, zone 3), where peroxisome proliferation starts initially, clearly indicating that the cell proliferation was the consequence of peroxisomal proliferation (Valles *et al.*, 2003; Kaufmann *et al.*, 2002).

Thus there are data from studies of DINP which satisfy the ILSI consensus criteria for peroxisomal proliferation. DINP produces liver tumors in rats and mice by a non-genotoxic process. All of the hallmark criteria for peroxisomal proliferation, *i.e.*, liver enlargement, peroxisome proliferation and cell proliferation, have been shown to occur in both rats and mice by at least three independent laboratories.

We note that, while DINP does meet the criteria from the 1995 ILSI workshop (Cattley *et al.*, 1998), the subsequent ILSI RSI workgroup update found that “the demonstration of PPAR α agonism was sufficient to abrogate the necessity for some of the more rigorous (and technically demanding) requirements determined by the previous working group” (Klaunig *et al.*, 2003, p. 687). DINP is one of examples of a PPAR α agonist used by the ILSI RSI workgroup to develop its conclusions (*e.g.*, Klaunig *et al.*, 2003, p. 667).

**3. The DINP Liver Tumors Meet the IARC Criteria for Irrelevance to
 Humans**

As stated above, IARC has reviewed the data on peroxisome proliferation and concluded that, when a tumor response in rats and mice is judged to be secondary to peroxisome proliferation, the substance may be considered as not presenting a carcinogenic risk to man (IARC, 1995). IARC has in fact applied these criteria to determine that liver tumors in rodents treated with a phthalate are not relevant to humans. In February 2000, an IARC working group met to consider carcinogenicity data and other evidence of peroxisome proliferation for DEHP. Based on mechanistic data and other information, IARC concluded that the mechanism by which DEHP increases the incidence of hepatocellular tumors in rats and mice is not relevant to humans (IARC, 2000). Although DINP has not yet been evaluated by IARC, the available data are very similar to those for DEHP, so similar conclusions are anticipated.

The criteria established by IARC to make the determination that the tumors are not relevant to humans are (IARC, 1995 at 12-13):

- (a) Information is available to exclude mechanisms of carcinogenesis other than those related to peroxisome proliferation.
- (b) Peroxisome proliferation (increases in peroxisome volume density or fatty acid β -oxidation activity) and hepatocellular proliferation have been demonstrated under the conditions of the bioassay.

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- (c) Such effects have not been found in adequately designed and conducted investigations of human groups and systems.

The data for DINP meet all of these criteria. With respect to the first criterion, alternative mechanisms of carcinogenicity, IARC relies substantially on the same types of information considered by ILSI, *i.e.*, is there evidence that peroxisomal proliferation does occur in the species which develop cancer, and, can a role for a genotoxic process be ruled out.⁵ (A genotoxic chemical is one that damages cellular DNA and may thereby trigger cancerous growth of the cell.) As described above, DINP does produce tumors in livers of rats and mice (Moore, 1998a; b), and there is clear evidence of peroxisomal proliferation in the livers of both species (Moore, 1998a; b; Smith *et al.*, 2000; Valles *et al.*, 2003; Kaufmann *et al.*, 2002). That DINP is not genotoxic is shown in Section II, above. In addition, there is no evidence of any pathologic changes in the livers of these species unrelated to peroxisome proliferation which could provide an alternative explanation for tumor formation (Lington *et al.*, 1997; Moore 1998a; b). Further, the electron microscopic evaluation in mice revealed exclusively findings related to peroxisome proliferation; no other degenerative findings on the subcellular level were observed in either sex (Kaufmann *et al.*, 2002).

Ito *et al.* (2007) have proposed an alternative mechanism for induction of liver tumors by another phthalate (DEHP) that is independent of PPAR α activation. As discussed in Section V.A.4.a, below, the utility of the mouse model employed by Ito *et al.* is limited, such that the data of Ito *et al.* are not sufficient to indicate there is a valid alternative mechanism of carcinogenesis other than that related to peroxisomal proliferation. Thus, the first IARC criterion is met.

The second criterion requires that peroxisome proliferation and hepatocellular proliferation be demonstrated under the conditions of the bioassay. As indicated above, increases in peroxisomal volume density, fatty acid β -oxidation, and hepatocellular proliferation in livers of rats and mice treated with DINP have been documented (Barber *et al.*, 1987; Moore, 1998a; b; Smith *et al.*, 2000; Valles *et al.*, 2003; Kaufmann *et al.*, 2002). In the rat study (Moore, 1998a), the tumors appeared only at the highest dose (1.2% in the diet or approximately 733 mg/kg/day in male rats and 885 mg/kg/day in females). As also documented in the laboratory report describing that study (Moore, 1998a), DINP also caused significant increases in liver weight, peroxisomal enzyme induction, and enhanced cell replication at that level. An independent study (Smith *et al.*, 2000) confirmed these observations at the same levels in the same strain of rats. Thus the requirement that peroxisomal proliferation be demonstrated under the conditions of the bioassay has clearly been met in rats.

In the Moore mouse study, liver tumors were significantly increased in male mice given 4000 or 8000 ppm (approximately 740 and 1560 mg/kg/day) and in female mice given 1500, 4000 or 8000 ppm (approximately 336, 910 and 1888 mg/kg/day) in the diet for two years (Moore, 1998b). As defined by the study protocol, liver weights, peroxisomal enzyme induction and cell replication were examined in only the high dose group (8000 ppm) and the control, and

⁵ See, *e.g.*, the IARC monograph discussion for DEHP (IARC, 2000, pp.116-121).

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all of these parameters were significantly elevated in the high dose group from that study (Moore, 1998b). An independent study also measured liver weight increase, peroxisomal enzyme induction, and enhanced cell replication in the same strain of mice treated at 6000 ppm (Smith *et al.*, 2000), and again all of these parameters were significantly elevated with respect to control. To evaluate peroxisome proliferation at the 1500 ppm and 4000 ppm levels, another study was conducted to determine the dose-response relationships for peroxisomal volume density and peroxisomal enzyme induction in mice treated with DINP. The data indicated that both peroxisome volume density and peroxisomal induction were significantly elevated at the tumorigenic doses (Kaufmann *et al.*, 2002). These new data provide direct evidence of peroxisomal proliferation under the conditions of the bioassay in the mouse as well as the rat. Taken together, these data demonstrate that, at every tumorigenic dose level in both rats and mice, there is a significant increase in peroxisome proliferation. Thus peroxisomal proliferation has been demonstrated under the conditions of the bioassay for DINP, meeting the second IARC criterion.

The third criterion requires evidence that peroxisome proliferation effects do not occur in “adequately designed and conducted investigations of human groups or systems.” For this, IARC normally relies on data from studies in primates and/or human hepatocytes in culture. There have been two studies in non-human primates; in one of these DINP had no effects on the liver and showed no other evidence of peroxisome proliferation in marmosets following 90 days of treatment at levels up to 2500 mg/kg/day (Hall *et al.*, 1999). In the other, DINP had no effects on the liver and showed no other evidence of peroxisome proliferation in cynomolgus monkeys following 14 days of treatment at levels up to 500 mg/kg/day (Pugh *et al.*, 2000). Similarly, there was no evidence of peroxisome proliferation in either human hepatocytes (Baker *et al.*, 1996; Hasmall *et al.*, 1999; Kamendulis *et al.*, 2002) or other primate hepatocytes tested under *in vitro* conditions (Benford *et al.*, 1986; Kamendulis *et al.*, 2002). Thus studies from several laboratories using hepatocytes from different individuals or different species of primates have demonstrated that a peroxisome proliferator response is not elicited by DINP in humans and other primates.

In summary, DINP meets all three IARC criteria for identifying a peroxisome proliferator for which liver tumors in rodents are not relevant to humans.

In 2000, IARC reviewed the evidence for DEHP in light of its criteria and determined that the classification of DEHP should be changed from Group 2B (probable human carcinogen) to Group 3 (not classifiable as to carcinogenicity). IARC summarized its determination for DEHP as follows:

In making its overall evaluation of the possible carcinogenicity to humans of di(2-ethylhexyl) phthalate, the working group took into consideration that (a) di(2-ethylhexyl) phthalate produces liver tumours in rats and mice by a non-DNA-reactive mechanism involving peroxisome proliferation; (b) peroxisome proliferation and hepatocellular proliferation have been demonstrated under the conditions of the carcinogenicity studies of di(2-ethylhexyl) phthalate in mice and rats; and (c) peroxisome proliferation has not

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been documented in human hepatocyte cultures exposed to di(2-ethylhexyl) phthalate nor in the livers of exposed non-human primates. Therefore, the mechanism by which di(2-ethylhexyl) phthalate increases the incidence of hepatocellular tumours in rats and mice is not relevant to humans.

(IARC, 2000, p. 124.)

As shown above, the data for DINP completely parallel those for DEHP.

- Like DEHP, DINP is not genotoxic (Barber *et al.*, 2000; McKee *et al.*, 2000; Zeiger *et al.*, 1985). It produces peroxisome proliferation in rodent liver (Barber *et al.*, 1987; Bird *et al.*, 1986; Bio/Dynamics, Incorporated, 1982; Moore, 1998a;b; Smith *et al.*, 2000; Kaufmann *et al.*, 2002), but does not produce such effects in PPAR α -deficient mice (Valles *et al.*, 2003).
- Peroxisomal proliferation and hepatocellular proliferation have been demonstrated under the conditions of the carcinogenic studies of DINP (Moore, 1998a; b; Smith, *et al.*, 2000; Kaufmann *et al.*, 2002; Valles *et al.*, 2003).
- Peroxisome proliferation has not been observed in cultured human hepatocytes treated with DINP or in hepatocytes from subhuman primates treated with DINP under both *in vivo* and *in vitro* conditions (Baker *et al.*, 1996; Benford, *et al.*, 1986; Hasmall, *et al.*, 1999; Hall *et al.*, 1999, Pugh *et al.*, 2000; Kamendulis *et al.*, 2002).

Therefore, for the same reasons IARC found that the liver tumors in rodents exposed to DEHP are not relevant to humans, the liver tumors observed in rats and mice exposed to high doses of DINP are not relevant for human risk assessment.

4. A PPAR α -Dependent Mechanism Is the Only Plausible Mechanism for the Liver Tumors

Not only is there evidence that DINP induces peroxisomal proliferation in rats and mice, there is also direct evidence that induction of the peroxisomal functions is related to activation of the PPAR α receptor. Clearly peroxisomal proliferation is the most plausible mechanism for the liver tumor response in rats and mice (Klaunig *et al.*, 2003). With respect to the question of whether there are any other processes that provide a plausible explanation for the tumors in rodents, there are three sub-questions: (a) Is there evidence for processes in rodents other than those associated with peroxisome proliferation which could explain the liver tumor response? (b) Is there plausible evidence that the consensus view of this carcinogenic process, *i.e.*, that it involves a PPAR α -mediated process, is inaccurate? and (c) Is there plausible evidence for an alternative process which could be operative in humans?

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- a. *Is there evidence for processes in rodents other than those associated with peroxisome proliferation which could explain the liver tumor response?*

Other mechanisms for carcinogenicity in rodents are not supported by the data. Since DINP is not genotoxic, the liver tumors could not have been initiated by a direct interaction between chemical and DNA. Therefore, the tumors must have been due to a secondary process related to cellular injury in the organ. There is no histologic evidence in the rodent studies for any liver changes other than those associated with peroxisomal proliferation. This was also confirmed by electron microscopy in mice, which revealed no other degenerative changes on the subcellular level (Kaufmann *et al.*, 2002). In particular, there was no evidence of any other compensatory cell proliferation resulting from a toxic process other than enhanced replicative DNA synthesis, a PPAR α -mediated process. DNA synthesis was statistically enhanced in the same hepatic compartment (perivenous, zone 3) where peroxisome proliferation was predominantly exhibited. There was evidence of inhibition of gap junctional intercellular communication (GJIC) (Smith *et al.*, 2000), but, as noted by IARC (1995), this is not inconsistent with a peroxisomal proliferation-mediated process. In fact, the ILSI RSI workgroup identified GJIC as a key event associated with the PPAR α mode of action (Klaunig *et al.*, 2003, p. 671). GJIC inhibition could act in concert with either enhanced cell replication or inhibition of apoptosis – which are the consequence of activation of the PPAR α receptor – facilitating the expression of tumors in rodents following peroxisomal proliferator treatment (McKee, 2000).

Ito *et al.* (2007) have proposed an alternative mechanism for induction of liver tumors by another phthalate (DEHP) that is independent of PPAR α activation. The report compares the effects of long-term dietary exposure of up to 0.05% DEHP on liver toxicity of wild type and PPAR α null (-/-) mice. The results presented must be carefully considered in light of the utility of the PPAR α null mouse model used and additional reports indicating an inherent susceptibility of these mice to tumorigenesis.

Ito *et al.* reported the use of a PPAR α -/- mouse strain produced according to a method published by Lee *et al.* (1995). These knockout mice had both PPAR α alleles replaced using the homologous recombination technique. Four biological endpoints were assessed after 24 months of treatment; the endpoints were referred to as: macroscopic liver findings; microscopic liver findings; oxidative damage (8-OHdG levels) and proto-oncogene expression levels (mRNA and/or protein). A statistically significant increase in the number of liver tumors (*i.e.*, hepatocellular carcinomas, hepatocellular adenomas, and cholangiocellular carcinomas) from 2-8 (10-25.8%) was seen between the wild type and knockout mice fed the top dose DEHP diet ($p < 0.05$). This was mostly due to a jump from 2 to 6 in hepatocellular adenoma incidence between these two groups. It should be noted that statistical significance was reached *only* when the total numbers of tumors were combined. Ito *et al.* discuss the low number of tumors and report them to be a reflection on the relatively low doses of DEHP used in the study.

There was no significant effect reported on bodyweight or liver weight, though the data suggested a trend towards an increase in liver weight for the PPAR α -/- animals, especially the 0.05% DEHP exposed group (+/+ mean = 1.27g \pm 0.18; -/- mean = 1.78g \pm 0.84). The reported 8-

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OHdG data suggest -/- mice suffered from an increased hepatic oxidative stress with DEHP as compared to the +/+ mice, though this was not supported by unchanged mRNA levels for 8-oxoguanine DNA-glycosylase 1, an 8-OHdG repair enzyme. As +/+ mice showed lower 8-OHdG levels than -/- mice at all DEHP exposure levels *and* in the controls (0% DEHP), PPAR α may prevent the oxidation of DNA dG. Ito *et al.* did not address the plausibility of any biological relevance between raised 8-OHdG levels and increased incidence of liver tumors in -/- mice exposed to DEHP.

On the basis of their data, Ito *et al.*, 2007 proposed an alternative mechanism for DEHP induced liver tumors, which is independent of PPAR α activation in that inflammation and protooncogenes altered by 0.05% DEHP-derived oxidative stresses may be involved in the tumorigenesis found in the PPAR α -null mice, but not in wild-type mice. However, the utility of these data is limited in that a number of reports have indicated that aged PPAR α null mice are more vulnerable to tumorigenesis due to fundamental mechanistic differences (Mandard *et al.*, 2004; Kostadinova *et al.*, 2005; Balkwill and Couseens, 2005; Pikarsky *et al.*, 2004; Takashima *et al.*, 2008). Most recently, gene expression profiles of hepatocellular adenoma tissues as well as control livers of wild-type and PPAR α null mice were examined (Takashima *et al.*, 2008). The genes identified to contribute to tumorigenesis (i.e. Gadd45a and caspase 3-dependent apoptosis genes) in the null mice were unique to the null mice.

As spontaneous tumors are known to occur in the PPAR α null mice at 24 months, Ito *et al.* indicate the possibility that DEHP merely promoted the formation of the spontaneous liver tumors in the aged null mice; a mechanism that is unique to the null mice and would not exist in the wild type mice. Importantly, with respect to DINP, literature searches reveal no reports that DINP induces production of reactive oxygen species in livers of rodents, humans or non-human primates, or in cultured liver cells from these species. Therefore, the Ito *et al.* (2007) data are not sufficient to indicate there is a valid alternative mechanism of carcinogenesis other than that related to peroxisomal proliferation.

- b. *Is there plausible evidence that the consensus view of this carcinogenic process, i.e., that it involves a PPAR α -mediated process, is inaccurate?*

There are four non-exclusive hypotheses to explain the carcinogenic effects of peroxisome proliferators; (i) that oxidative stress related to induction of peroxisomal enzymes leads to malignant transformation, (ii) that enhanced replicative synthesis facilitates the expression of these (or spontaneously) transformed cells, (iii) that inhibition of apoptosis prevents transformed cells from being removed by normal homeostatic mechanisms, and/or (iv) these in combination (Peters *et al.*, 2000). The sufficiency of these processes to explain the carcinogenic response is consistent with current theoretical models. The empirical evidence comes from a study in which a mouse strain lacking PPAR α did not have elevated levels of peroxisomal enzymes or enhanced cell replication and did not develop liver tumors following treatment with a potent peroxisome proliferating agent (Peters *et al.*, 1997). An alternative proposal was that Kupffer cells initiated the proliferation response through production of tumor necrosis factor alpha (TNF α) by a process independent of PPAR α (Rose *et al.*, 1999). However,

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more recent data has shown that rodent liver hepatocytes respond to Kupffer cell-derived TNF α through mechanisms dependent on expression of PPAR α in parenchymal cells (Peters *et al.*, 2000), and the ILSI RSI workgroup identified Kupffer cell-mediated events as a key event associated with the PPAR α mode of action (Klaunig *et al.*, 2003, p. 671). Thus there is no plausible explanation for the rodent liver tumors except a PPAR α -mediated process.

c. *Is there plausible evidence for an alternative process which could be operative in humans?*

Gonzalez *et al.* (1998) concluded that all peroxisome proliferators are likely to cause tumors through activation of PPAR α , and not via other nuclear receptors, including PPAR β or PPAR γ . The activity of PPAR α is not the same in humans as in rodents. As described in more detail below, there is only one function related to PPAR α activation in rodents which is also expressed in humans – fatty acid metabolism – and that proceeds by different pathways in these species. As reviewed by Vameq and Latruffe (1999), PPAR γ is involved in adipocyte differentiation, formation of foam cells and interference with tumor growth. Thus, activation of PPAR γ seems more likely to be involved in tumor protection than tumor induction. Further, in contrast to PPAR α , the activity of this receptor seems to be conserved across species. PPAR β may be involved in adipocyte differentiation but is not well understood. There is no plausible evidence that differences between humans and rodents could lead to an increased risk of cancer to humans. Again, this is demonstrated empirically. In studies of primate and human hepatocytes in culture, DINP does not produce peroxisome-proliferation related effects (Benford *et al.*, 1986; Baker *et al.*, 1996; Hasmall *et al.*, 1999; Kamendulis *et al.*, 2002). In primate *in vivo* studies, high doses of DINP do not produce liver changes of any kind. Pugh *et al.* (2000) performed a 14-day study of cynomolgus monkeys in which no liver effects – including no change in hepatic peroxisome β -oxidation – were observed from high doses of DEHP and DINP (500 mg/kg/day). Hall *et al.* (1999) reported that administration of 2500 mg/kg/day DINP to marmosets for 13 weeks produced no pathological changes in liver, kidneys or testes, and no evidence of peroxisomal proliferation.

* * * * *

In summary, for the reasons given above, there is no mechanism other than a PPAR α process that provides a plausible mechanism for the liver tumors observed in DINP-treated rodents.

5. The PPAR α Mechanism Does Not Operate in Humans

Having established that the mechanism by which DINP causes liver tumors is PPAR α -mediated, one could ask whether there is a theoretical possibility that tumors could arise in humans as a consequence of a peroxisome proliferation-mediated response. The evidence indicates that the answer to this question is no.

The demonstration that activation of PPAR α was an absolute requirement in the induction of liver carcinogenesis (Peters *et al.*, 1997) established a basis for species differences;

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levels of PPAR α in humans are substantially lower than they are in rodents. Palmer *et al.* (1998) have shown that humans have less than one-tenth the level of PPAR α expression observed in mice. These reduced levels appear to be the result of lower transcription rates, inefficient pre-messenger RNA splicing, or both (Palmer *et al.*, 1998; Tugwood *et al.*, 1996).

In addition to the reduced levels of PPAR α in humans, there is strong evidence that there are additional factors which prevent the expression in humans of the PPAR α -mediated functions which play a role in rodent cancer. Woodyatt *et al.* (1999) showed that, although human PPAR α could bind peroxisome proliferating agents (PP-agents) and that this complex could drive transcription of the acetyl co-enzyme A (ACO) in mouse cells, it could not drive transcription of this gene in human cells. In fact, the activity of the PPAR α /PP-agent complex may be a basis for species differences in metabolism of fatty acids: in rodents fatty acid metabolism involves activation of PPAR α by a PP-agent and transcription of the ACO complex, whereas in humans the PPAR α /PP-agent complex binds to a different response element and transcribes the apoA1 and apoCIII regions (summarized in Roberts, 1999).⁶ Vanden Huevel (1999) noted that there was interindividual variability in human PPAR sequences and wondered whether this could lead to individuals at increased risk. However, the identified human PPAR α variants have been either inactive (Woodyatt *et al.*, 1999) or dominant negative suppressors (Gervois *et al.*, 1999). Thus, the interindividual variability which has been identified has tended to reduce effective PPAR α levels in humans rather than to increase them. Further, Lawrence *et al.* (2001) tested this hypothesis directly with human cell lines (HepG2 cells) that “over-expressed” human PPAR α . They found that the PPAR α -related functions were not increased by PPAR α agonists, demonstrating that, although PPAR α is present in human cells, higher PPAR α levels, if present, could not lead to greater risk.

Thus, all of the available data indicate that there are both quantitative and qualitative differences between rodents and humans. The data shows that the levels of PPAR α in humans are at least an order of magnitude below those found in rodents. Further, although some fraction of human PPAR α can bind agonists and is active when tested with rodent receptors, the evidence suggests that it does not lead to transcription of similar functions in humans.

⁶ In rodents, lipid metabolism is mediated by peroxisomal enzymes, specifically acetyl CoA oxidase (ACO), whereas human lipid metabolism is mediated through alterations in gene expression of the major high density apolipoproteins, apoA1, apoAII and apoCIII as well as lipoprotein lipase (LPL) (reviewed in Vamecq and Latruffe, 1999). Roberts and coworkers (Lambe *et al.*, 1999; Woodyatt *et al.*, 1999) have shown that the human peroxisome proliferation response element (PPRE) differs in sequence from that of the rat. They have shown further that whereas both human and mouse PPAR α can drive transcription of mouse ACO, neither can drive transcription of the human ACO gene sequence (Woodyatt, *et al.*, 1999). Conversely, there are also differences between humans and rats in the sequence of the ApoA1 gene promoter; the human gene is activated by hypolipidemic agents whereas the rat gene sequence is not (Vu-Dac *et al.*, 1998). Thus, the lack of expression of residual peroxisomal function in primates and cultured human cells seems to be a consequence of differences between humans and rats at the transcriptional level in control of lipid metabolism.

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There is also inferential evidence that the PPAR α -related functions related to rodent liver carcinogenicity are not expressed in humans. A review article by Gonzalez *et al.* (1998) noted that the mechanisms of rodent liver carcinogenicity associated with peroxisome proliferation included oxidative stress (which the authors associated with expression of peroxisomal enzyme induction) and enhanced cell proliferation. They also believed there to be a role for apoptosis (programmed cell death, inhibited by peroxisome proliferators) and tissue necrosis factor α (TNF- α), a hepatocyte growth factor secreted by Kupffer cells. They reported that humans differed from rodents in expression of PPAR α -related functions in a number of ways (Table 3).

Table 3.
 Comparison of Human and Rodent Expression of PPAR α -Related Functions
 (from Table 2 in Gonzalez *et al.*, 1999)

Response to peroxisome proliferators	Mice and Rats	PPAR α -Null Mice	Humans
PPAR α expression	+	-	+/10 (10 fold less than mice)
increase in peroxisomes	+	-	-
enzyme induction	+	-	-
cell proliferation	+	-	-
apoptosis inhibition	+	-	? [see text]
hypolipidemic effects	+	-	+
anti-inflammatory effects	+	-	? [see text]
increased risk of cancer	+	-	-

Since the publication of that table, the two question marks in the human column have been answered. Apoptosis in human hepatocytes has been shown to be unaffected by DINP (Hasmall *et al.*, 1999), and PPAR α activation seems to have no role in inflammatory processes in humans (Vameq and Latruffe, 1999). In addition, the positive hypolipidemic effects in humans have been shown to occur by a process which is different from that which is active in rats and mice (Vameq and Latruffe, 1999).

Thus the most plausible interpretation consistent with the data is that the PPAR α -mediated functions associated with carcinogenic induction in mice and rats are not expressed in humans. A large body of empirical evidence which is consistent with that view supports this assertion. Hall *et al.* (1999) showed that DINP treatment could not induce peroxisomal proliferation and had no effects on levels of peroxisomal enzymes in marmosets, at levels well above those associated with effects in rats and mice. These results were confirmed by Pugh *et al.* (2000) through studies with cynomolgus monkeys. Similarly under *in vitro* conditions, DINP increased replicative DNA synthesis and suppressed apoptosis in rodent hepatocytes but had no effects in human cells (Hasmall *et al.*, 1999), and MINP, the monoester corresponding to DINP, had no effects on peroxisomal enzyme levels in either human or primate hepatocytes in culture (Benford *et al.*, 1986; Kamendulis *et al.*, 2002).

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**6. Even if DINP did Cause Peroxisome Proliferation in Humans,
Human Internal Dose Levels Cannot Reach Carcinogenic Levels**

The foregoing makes clear that the liver tumors observed in rodents treated with DINP simply are not relevant to humans. However, even assuming it were possible for DINP to cause some peroxisome proliferator response in humans, there is no conceivable scenario under which humans could be exposed to sufficient amounts of DINP to cause liver tumors, for two reasons. First, because of differences between primate and rodent absorption of DINP, internal doses equivalent to those required to produce tumors in rodents simply cannot be achieved in humans. Second, even if sufficiently high internal doses could somehow be achieved, actual exposures to such doses of DINP would not occur under any plausible scenario.

The ILSI RSI workgroup concluded that, for PPAR α agonists in general, taking into account kinetic and dynamic factors, the animal mode of action is not plausible in humans (Klaunig *et al.*, 2003, pp. 691-693). This is specifically demonstrated by phthalate data on differences in absorptive capacity between rodents and primates, which demonstrate that the relatively high internal doses associated with effects in rodents cannot be achieved in humans.

The rodent data indicate that approximately 50% of orally administered DINP is absorbed as the corresponding monoester at dose levels up to 500 milligrams per kilogram per day (mg/kg/day) (Lington *et al.*, 1985; El-Hawari, *et al.*, 1985; 1983). Data from studies of absorption of DEHP in rodents indicate that this relationship is preserved at even higher treatment levels (Rhodes *et al.*, 1986). Primates, however, respond very differently. Data from studies with DEHP in both the marmoset (Rhodes *et al.*, 1986) and cynomolgus monkeys (Astill, 1989) show that, even at very high treatment levels, absorption in the primates is limited and that internal doses do not exceed those measured in rats exposed to 150-200 mg/kg/day. Comparative dosimetry studies (Pugh *et al.*, 2000) indicate that DINP is even more poorly absorbed by primates than DEHP. Studies with volunteers also indicate that humans absorb a much lower fraction of the dose than rodents (Anderson *et al.*, 2001). These data emphasize that consideration of the likely internal dose, based on toxicokinetic considerations, is crucial to an evaluation of the potential for toxicological effects in humans from DINP exposures. The data indicate that effects produced in rodents by DINP will not occur in humans, at least in part because the high internal doses required to produce these effects in rodents cannot be achieved in humans.

The lowest DINP dose that has been associated with tumor induction is 336 mg/kg/day in female mice with effects in other species and sexes occurring at levels ranging from approximately 700 to 900 mg/kg/day (Moore *et al.*, 1998a; b). As stated above, the maximum level absorbed by primates corresponds to a rodent level of 150-200 mg/kg, well below the dose required to induce tumors in the more sensitive rodents. Thus, the evidence indicates that, regardless of the level of exposure, humans could never absorb enough DINP to achieve the internal doses associated with liver tumors in rodents. That the doses which can be achieved in humans would not pose any concern is indicated by the fact that 2,500 mg/kg/day for 13 weeks produced no liver effects whatsoever in marmosets (Hall *et al.*, 1999).

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Even if human doses were not limited by absorptive capacity, however, exposure to the amount of DINP that would be required to cause liver tumors simply would not occur under any plausible exposure scenario. As stated above, the lowest level at which DINP produced a carcinogenic response was 336 mg/kg/day (female mice). If one were to assume that humans and mice were equally sensitive, a 70 kg human would have to ingest an average of 23,500 mg/day over an entire lifetime to achieve a dose to that which caused liver tumors in mice.

However, humans and rodents are not equally sensitive. As described above, the levels of PPAR α in humans are at least an order of magnitude below those found in rodents (Tugwood *et al.*, 1996; Palmer *et al.*, 1998). Thus, humans should be at least an order of magnitude less sensitive than animals. That brings the required dose to approximately 235,000 mg/day for a 70 kg adult (or approximately half a pound per day of undiluted material). (In fact, the required dose would be yet higher, because, as discussed above, human PPAR α does not respond to DINP in the same manner as rodent PPAR α .) Such high doses simply cannot be reasonably foreseen for humans.

Since 1999, the CDC has been analyzing samples of urine from the U.S. population for phthalate metabolites. CDC has reported its biomonitoring findings in reports issued in 2001, 2003 and 2005. The 2003 report includes the data from the 2001 report, and provides results for samples from 2541 persons (CDC, 2003). The 2005 report provides data for an additional 2772 persons (CDC, 2005). The DINP exposure corresponding to the urinary metabolite concentration can be calculated using the method of David (2000).

For the results reported in 2003, no DINP metabolite was detected at the 50th or 75th percentile levels. At the 95th percentile, the creatinine-corrected value for the total population was 4.29 $\mu\text{g/g}$, which corresponds to a DINP exposure of 0.88 $\mu\text{g/kg/day}$. The 95th percentile creatinine-corrected value for children, age 6-11, was 6.00, corresponding to a DINP exposure of 0.67 $\mu\text{g/kg/day}$.⁷

For the results reported in 2005, no DINP metabolite was detected in the overall population even at the 95th percentile, nor in subgroups divided by age. It was detected at the 95th percentile for Mexican Americans and Non-Hispanic Blacks, although the levels reported were lower than those reported in 2003. The higher of the two 95th percentile levels was for Mexican Americans – 2.31 $\mu\text{g/g}$. That converts to an exposure of 0.67 $\mu\text{g/kg/day}$.

Conservatively assuming a 70 kg person were exposed to the 2003 95th percentile level of exposure every day of their life, that person's exposure would be approximately 62 $\mu\text{g/day}$ – a level that is approximately 3,800,000 times less than 235,000 mg/day, the minimum shown above that would be required to cause tumors (assuming human PPAR α could respond to DINP in an equivalent manner to rodent PPAR α).

⁷ Restrictions on use of DINP in toys and child care products became effective in 2009 under both California and Federal law. Thus, future exposures to DINP will be even less, particularly for children.

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Thus, it is not plausible that exposures of Californians to DINP would contribute to cancer incidence.

* * * * *

In summary, there is strong evidence that the PPAR α mechanism which is responsible for liver tumors in DINP-treated rodents is not operable in humans. Even if PPAR α in humans did respond to DINP in a manner similar to rodent PPAR α , it simply is not possible for humans to achieve sufficient doses of DINP to result in liver tumors. This is because actual exposure to DINP, even under extreme scenarios, is far below the doses that would be required and because, even if humans were to receive high doses of DINP, they would not absorb sufficient DINP to result in internal doses high enough to lead to liver tumors. Thus, DINP cannot be reasonably anticipated to cause liver cancer in humans.

7. Expert Body Reviews Have Concluded that the Rodent Liver Tumors in DINP Studies Are Not Relevant to Humans

The CPSC CHAP concluded “that DINP causes liver cancer in rodents by a PPAR α -mediated mechanism, that is pronounced in rodents and believed not readily induced in humans, especially at doses resulting from current use of consumer products” (CPSC, 2001, p. 122). Subsequently, the CPSC staff, based on the CHAP and on the ILSI workshop, have “concluded that DINP, which is a peroxisome proliferator, is not likely to present a cancer risk in humans” (CPSC, 2003).

The ILSI RSI workgroup concluded:

In summary, the weight of evidence overall currently suggests that the rodent [mode of action] for liver tumors is not likely to occur in humans, taking kinetic and dynamic factors into account. This conclusion is based upon evaluation of the existing body of evidence and would apply to the consequences of exposure to *known* examples of PPAR α agonists.

(Klaunig *et al.*, 2003, p. 693.) DINP is a known example of a PPAR α agonist that was part of the basis for the workshop conclusions. Therefore, the conclusion of the ILSI workshop is that the liver tumors that occur in rodents treated with DINP are not likely to occur in humans.

The EU in its risk assessment of DINP stated:

The current literature suggests that only rats and mice are responsive to the carcinogenic effects of peroxisome proliferator, while dogs, non-human primates and humans are essentially non-responsive or refractory. In this way, it should be noted that in monkey, following oral administration of DINP for 14 days or 13 weeks there was no evidence of peroxisome proliferation. This indicates that monkeys and subsequently probably humans are far

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less sensitive than rodents to peroxisome proliferation and its relative liver effects. It should be noted that recently IARC gave a ruling on the carcinogenicity of DEHP and concluded that the mechanism (peroxisome proliferation and PPAR α activation) by which DEHP increased the incidence of liver tumours in rodents was not relevant to humans. (ECB, 2003a, p. 243)

The EU did not identify carcinogenicity as a critical endpoint (ECB, 2003a, 2003b) and has not classified DINP as a carcinogen (EC, 2000). In the risk assessment summary document, the EU stated that, on the basis of the peroxisome proliferation evidence, "there is no concern for a potential carcinogenic effect in humans." (ECB, 2003b, p. 14)

When EPA originally proposed to list DINP under EPCRA Section 313 (Fed. Reg. 2000), the American Chemistry Council requested that several prominent researchers provide opinions on the potential human carcinogenicity. Those opinions were provided in comments submitted to EPA in 2001; copies as provided with these comments, as follows:

- Attachment A is a statement by Ruth Roberts, Ph.D., Head of Cell Biology Research and Cancer Project Manager at Syngenta Central Toxicology Laboratory in the United Kingdom. She holds a Doctorate in Medical Oncology and completed a Postdoctoral Fellowship in molecular oncology. Dr. Roberts has performed some of the foremost research on the mechanism by which peroxisome proliferators cause cancers in rodents and whether that mechanism operates in humans. Dr. Roberts concludes: "weight of the evidence supports the position that the rodent liver tumors caused by peroxisome proliferators such as DINP are not relevant to man since we differ from rodents at the molecular level in our response to peroxisome proliferators."
- Attachment B is a statement by James Klaunig, Ph.D. Dr. Klaunig is Professor and Director of Toxicology at the Indiana University School of Medicine and is Director of the State Department of Toxicology for the State of Indiana. He holds his Doctorate in Experimental Toxicology/Pathology and has done Postdoctoral work in pathology. He serves and has served on numerous review committees for government agencies, including EPA, NTP and NIH. Dr. Klaunig has conducted significant research on peroxisome proliferation mechanisms and participated in the ILSI RSI workshop on peroxisome proliferators. He concludes that the data "provide mechanistic evidence that rodent liver tumor induction by DINP is by a peroxisomal proliferation process which does not occur in humans or other primates." [Note that the "unpublished data" provided with Dr. Klaunig's statement has now been published (Kamendulis *et al.*, 2002).]
- Attachment C is a cancer risk assessment for DINP by Gary Williams, M.D., and Michael Iatropoulos, M.D., Ph.D. Dr. Williams is Professor of Pathology, Director of Environmental Pathology and Toxicology, and Head of the Program on Medicine, Food and Chemical Safety, at the New York Medical College. Dr. Williams is a recognized expert in chemical carcinogenesis; Dr. Iatropoulos is a Research Professor

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of Pathology at New York Medical College and is also an expert in chemical carcinogenesis. Drs. Williams and Iatropoulos reviewed the data for DINP with respect to liver and kidney tumors and MNCL. They concluded, "the increases in all three spontaneously occurring tumors seen with DINP occurred through processes not relevant to humans and at exposures vastly beyond that which would take place with product use."

In summary, numerous independent scientists have evaluated the potential for peroxisome proliferators in general or DEHP and DINP in particular to cause cancer in humans. The overwhelming scientific consensus is that DINP cannot reasonably be anticipated to cause cancer in humans.

B. Mononuclear Cell Leukemia Observed in Fisher 344 Rats Is Not Relevant to Humans

Mononuclear cell leukemia (MNCL) was observed in the two DINP bioassays conducted in Fisher 344 rats, but not in the bioassay conducted in mice (Lington *et al.*, 1997; Moore, 1998a; b). MNCL is a lesion that occurs almost exclusively in the F-344 rat, and that occurs spontaneously in that species. MNCL is discounted by authoritative agencies such as the National Toxicology Program (NTP) and the International Agency for Research on Cancer (IARC). As described below and in the attached opinion from Dr. Richard Irons (Attachment D), a preeminent researcher of leukemogenesis, the use of MNCL as a basis for human health risk assessment is not scientifically supportable. In fact, Dr. Irons notes that a proposal he made to the National Institutes of Health (NIH) had been rejected because of the "obvious lack of significance of MNCL to human disease."

1. MNCL in Fischer Rats Is Generally Disregarded for Human Risk Assessment

MNCL is a spontaneous tumor which occurs frequently in the F-344 rat and is the most common cause of spontaneous death in that strain and species (*e.g.*, Haseman *et al.*, 1998). NTP historical control data show that MNCL occurs in 14 to 74 percent of control animals (Haseman *et al.*, 1998). Background incidence is seen to be highly variable and has more than doubled during the two decades since the Haseman *et al.* report in 1985. (Thomas *et al.*, 2007). MNCL is found at much lower incidence in other rat strains (Iatropoulos, 1983) and has not been reported in mice (*e.g.*, Harleman *et al.*, 1994). There may also be differences within strains – the incidence of MNCL seems much lower in Japanese F-344 rats than in those used by the NTP (Whysner *et al.*, 1995).

The results of DINP chronic studies are consistent with these findings. MNCL was found in two studies in the F-344 rat (Lington *et al.*, 1997; Moore *et al.*, 1998a) but not in the B6C3F1 mouse (Moore *et al.*, 1998b) or the Sprague-Dawley rat (Bio/dynamics, 1986).

When assessing the significance of changes in MNCL incidence, points to consider include: (1) that the factors contributing to a high, variable, spontaneous incidence of MNCL in the F-344 rat are unknown; (2) that there are a number of factors which contribute to variability

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in MNCL frequency for unknown reasons – including the use of corn oil as a vehicle (Haseman *et al.*, 1985), single vs. group housing (Haseman *et al.*, 1998), splenic toxicity, lifespan, body weight and dietary fat (but not dietary restriction) (Elwell *et al.*, 1996); and (3) that treatment with genotoxic agents that might logically be expected to increase the incidence of cancer in general have either no effect or actually reduce MNCL incidence (Waalkes *et al.*, 1991; Lijinsky *et al.*, 1993; Elwell *et al.*, 1996).

Many authoritative sources have questioned the relevance of MNCL data for human risk assessment purposes. For example, the NTP, in its review of the carcinogenesis data for diallyl phthalate wrote:

The relatively high and variable spontaneous incidence of mononuclear cell leukemia in aged F-344 rats confounds the interpretation of this tumor type in dosed animals as evidence of a carcinogenic response. That is, statistical evidence of an increased occurrence of mononuclear cell leukemia in dosed animals as an indication of carcinogenicity may appropriately be regarded with less confidence than would similar incidence data for other tumor types in the F-344 rat. (NTP, 1984).

In a review of tetrachloroethylene, the United Kingdom Health and Safety Executive (HSE) noted that MNCL was a common neoplasm that occurred at high and variable frequency in the F-344 rat. They did not consider an excess of MNCL as evidence for a carcinogenic response even though the frequency exceeded the historical averages of both the NTP and the testing laboratory (HSE, 1987). As noted above, NIH rejected a proposal by Dr. Irons because of the “obvious lack of significance of MNCL to human disease.”

EPA has not generally regarded MNCL in Fisher 344 rats to be indicative of any human cancer concern. For example, EPA reviewed the Lington DINP study, including the MNCL data, and concluded that “no evidence of carcinogenicity has been found in these studies” (Hirzy, 1989). Additionally, in its review of butylbenzyl phthalate, EPA stated that the available evidence, including increased MNCL in F-344 rats, “does not indicate that BBP causes or can reasonably be anticipated to cause cancer in humans” (EPA, 1987).

In his opinion (Attachment D), Dr. Richard Irons, a pre-eminent researcher in the field of leukemogenesis, states, “In my view, MNCL in the F344 rat is not a useful model for the direct study of human disease and is certainly not an appropriate endpoint for predicting or extrapolating carcinogenic risk in humans,” and “there is no biologic rationale for concluding that F-344 MNCL is a relevant surrogate for a comparable disease entity or, independently, any disease that has been associated with chemical exposure in humans.” Dr. Irons reviewed the Lington and Moore data and concluded that “specifically with respect to bioassays of di-isononyl phthalate, the dose-dependent nature of treatment-related MNCL is not impressive, suggesting that the observed increases represent a non-specific high dose effect that cannot be meaningfully attributed to a carcinogenic event.”

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A recent review of MNCL (Thomas et al., 2007) suggests that a weight of evidence approach be taken when statistically identified increases in MNCL occur with exposure. The authors propose similarities between F344 MNCL and human NK-LGL leukemia based on functional, clinical and morphological characteristics, but emphasize that the mechanisms of leukemogenesis may be very different. Without further research to clarify the leukemic cell of origin, and define candidate molecular targets, the case for potential human relevance remains weak, particularly in light of the high, variable spontaneous incidence of MNCL in the Fischer 344 rat – the species in which MNCL was seen in conjunction with DINP administration.

**2. Expert Body Reviews Have Concluded that the MNCL
in DINP Studies Is Not Relevant to Humans**

The CPSC CHAP concluded:

The findings of mononuclear cell leukemia and renal tubular carcinoma in the rodent bioassay for DINP are of questionable relevance to humans. (CPSC, 2001, p. 122).

The EU Risk Assessment states:

Regarding MNCL, a clear increase incidence is observed in the two studies conducted with Fisher rats (outside the historical range of spontaneous leukemia), along with shortening of the onset of MNCL. However, MNCL is a common neoplasm in the Fischer 344 rats and the increased incidence after chronic exposure to some substances is likely a strain specific effect with little relevance for humans. Of interest, the IARC categorised MNCL as “an unclassified leukemia with no known human counterpart” and substances which increase MNCL frequency as “not classifiable as to carcinogenicity in humans” (IARC, 1990). (ECB, 2003a, p. 225).

Thus, the opinion of many authoritative bodies and the current literature continue to support the position that MNCL is not relevant for human health assessment. In addition, the CPSC CHAP, the EU, and Dr. Irons have specifically found that MNCL in the DINP bioassays is not relevant for human health assessment.

**C. The Mechanism for Formation of Kidney Tumors in Male Rats Exposed
to DINP Is Not Relevant to Humans**

Kidney tumors have been observed in male rats exposed to high doses of DINP for two years (Moore, 1998a), but not in female rats and not in mice of either gender (Moore, 1998a; b). Male rats are known to be susceptible to formation of kidney tumors through a mechanism involving alpha_{2u}-globulin accumulation. Because humans do not produce alpha_{2u}-globulin, such male rat kidney tumors are not relevant for human health assessment (EPA, 1991; Swenberg and Lehman-McKeeman, 1998). The kidney tumors observed in the DINP study were malignant

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tubule cell carcinomas, found in male rats given high dietary doses but not in female rats or in mice of either sex. See Table 4. The tumors found were of a type associated with an alpha_{2u}-globulin process and also demonstrated the sex- and species-specific responses expected for an alpha_{2u}-globulin process.

In the DINP study in rats, there was evidence in the male rats of microscopic changes characteristic of alpha_{2u}-globulin induction (Moore, 1998a). Subsequent studies have demonstrated that all the criteria established by the EPA and by IARC to verify that a carcinogenic response is the consequence of the alpha_{2u}-globulin mechanism are met for DINP (Caldwell *et al.*, 1999; Schoonhoven *et al.*, 2001). Attachment E is a letter from Dr. James Swenberg who is an expert in the alpha_{2u}-globulin mechanism (he is a co-author of the IARC scientific publication on the alpha_{2u}-globulin mechanism) and who has conducted some of the research on DINP. As stated by Dr. Swenberg, the data "clearly demonstrate that DINP causes [alpha_{2u}-globulin nephropathy]" and that "the data on kidney tumors is not relevant for human risk assessment."

Table 4.
Incidence of malignant tubule cell carcinomas in rats and mice following chronic dietary administration of DINP – number of rats per dose group (mg/kg/day)

	control	~30	~90	~400	~800	recovery *
male rats	0	0	0	0	2	4
female rats	0	0	0	0	0	0
	control	~100	~300	~800	~1600	recovery *
male mice	0	0	0	0	0	0
female mice	0	0	0	0	0	0

* Animals in the recovery group were given the high dose for 18 months and then held without treatment until terminal sacrifice (24 months).

1. The DINP Data Meet EPA's Criteria for an Alpha_{2u}-Globulin Mechanism

In 1991 the EPA reviewed the evidence for alpha_{2u}-globulin accumulation as a potential mechanism of renal cancer and its relevance to humans (EPA, 1991). This review culminated in a two part EPA science policy statement (EPA, 1991, p. 85):

(1) Male rat kidney tumors arising as a result of a process involving [alpha_{2u}-globulin] accumulation do not contribute to the qualitative weight-of-evidence that a chemical poses a human carcinogenic hazard. Such tumors are not included in dose-response extrapolations for the estimation of human carcinogenic risk.

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(2) If a chemical induces [α_{2u} -globulin] accumulation in male rats, the associated nephropathy is not used as an endpoint for determining non-carcinogenic hazard. Estimates of non-carcinogenic risk are based on other endpoints.

EPA also provided guidance for determining whether the α_{2u} -globulin process could be a factor in renal effects. Each of three factors, set forth in Section XVII-A of EPA (1991, pp. 86-87) must be met. As the following shows, all three factors are met for DINP.

"(1) Increased number and size of hyaline droplets in renal proximal tubule cells of treated male rats

The abnormal accumulation of hyaline droplets in the P2 segment of the renal tubule is necessary to attribute the renal tubule tumors to the [α_{2u} -globulin] sequence of events. This finding helps differentiate the [α_{2u} -globulin] inducers from chemicals that produce renal tubule tumors through other means." (EPA, 1991, p. 86)

As shown in Caldwell *et al.* (1999), hyaline droplets were evaluated by immunohistochemical staining (a process specific for α_{2u} -g) in male and female rats. Droplets were present in male rat kidneys, and both droplet size and area involved were significantly increased with dose. Droplets were not present in kidneys from female rats. The accumulation of α_{2u} -g in male rat kidneys with increasing dose was independently confirmed by a second laboratory (Schoonhoven *et al.*, 2001). These data demonstrate the abnormal accumulation of hyaline droplets in the renal proximal tubules of treated rats and show also that this does not occur in female rats, thus demonstrating the sex specificity of this finding.

"(2) Accumulating protein in the hyaline droplets is [α_{2u} -globulin]

Hyaline droplet accumulation is a nonspecific response to protein overload in the renal tubule and may not be due to [α_{2u} -globulin]. Therefore, it is necessary to demonstrate that [α_{2u} -globulin] accounts for the hyaline droplet accumulation found in the male rat." (EPA, 1991, p. 86)

As shown above, the evaluation of hyaline droplets utilized immunohistochemistry to detect the highly specific binding of a monoclonal antibody to α_{2u} -globulin. As documented by both Caldwell *et al.* (1999) and Schoonhoven *et al.* (2001), the accumulating protein in the hyaline droplets is α_{2u} -globulin. As stated above, the absence of α_{2u} -globulin in kidneys from female rats was also demonstrated, confirming the sex specificity of the observation.

"(3) Additional aspects of the pathological sequence of lesions associated with [α_{2u} -globulin] nephropathy are present.

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Typical lesions include single cell necrosis, exfoliation of epithelial cells into the proximal tubular lumen, formation of granular casts, linear mineralization of papillary tubules, and tubule hyperplasia. If the response is mild, all of these lesions may not be observed; however, some elements consistent with the pathological sequence must be demonstrated to be present." (EPA, 1991, pp. 86-87)

As documented in Caldwell *et al.* (1999), tubular regeneration and tubular epithelial hyperplasia were present in male rat kidneys, predominantly in the P2 segment of the proximal tubule of the renal cortex, and increased in a dose-responsive manner. In contrast, tubular regeneration was present in only one of the high dose female rats. Mineralization was documented in the pathology reports of the chronic studies (Moore, 1998a; b). This also showed a strong dose response relationship in the male rat kidneys. Mineralization was present in kidneys of some female rats but did not increase with dose, and was not present in kidneys of mice (Table 5). Lington *et al.* (1997) reported a statistically significant increase in renal epithelial cells in the urine. This is the consequence of exfoliation of epithelial cells into the proximal tubular lumen. Single cell necrosis and formation of granular casts were not reported, but as DINP is clearly a weak inducer of α_{2u} -g, all of the histological changes are not to be expected, and the absence of some, as noted by the EPA, is not inconsistent with an [alpha_{2u}-globulin] mediated response.

Table 5.
 Incidence of kidney mineralization following dietary administration of DINP.
 No. affected rats/total no. rats in each dose group (mg/kg/day)

	control	~30	~90	~400	~800	recovery *
male rats	16/60	14/50	11/50	59/60	57/60	50/50
female rats	11/60	9/50	4/50	14/50	16/60	10/50
	control	~100	~300	~800	~1600	recovery *
male mice	NP	NP	NP	NP	NP	NP
female mice	NP	NP	NP	NP	NP	NP

*Animals in the recovery groups were treated with the high dose for 18 months and then held without further treatment until terminal sacrifice (24 months).

NP – not present.

In a dietary study of DINP in Sprague-Dawley rats at levels of 0.3 and 1.0% for 13 weeks, tubular regeneration, nephritis, tubular casts and nephrosis were observed primarily in male rats, and increasing with dose (Bird *et al.*, 1986; Bio/Dynamics, 1982). These lesions are consistent with [alpha_{2u}-globulin] pathology and provide further evidence that the α_{2u} -g process was operative in causing the kidney tumors in male rats treated with DINP. Additionally, the appearance and extent of these lesions at 13 weeks further differentiate them from those associated with chronic progressive nephropathy, providing further evidence they are the consequence of an [alpha_{2u}-globulin] mediated process.

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Thus, all three of EPA's obligatory criteria are met for DINP. When this is the case, then EPA's guidance states that additional information is reviewed (EPA, 1991, Section XVII-B, pp. 87-88). Data are available for several of the categories of EPA describes,⁸ as follows:

- (a) Additional biochemical information (including reversible binding of the chemical to alpha_{2u}-globulin): As documented by Schoonhoven *et al.* (2001), reversible binding of DINP metabolites to alpha_{2u}-globulin has been demonstrated.
- (b) Sustained cell division in the proximal tubule of the male rat: This was documented by Caldwell *et al.* (1999) through the use of immunochemical techniques -- specifically, the use of the proliferating-cell nuclear antigen (PCNA) -- and was subsequently confirmed by Schoonhoven *et al.* (2001) through the use of an alternative technique -- BrdU labelling.
- (c) Genotoxicity (*i.e.*, information on potential genotoxicity in a standard battery of short-term tests relevant to the evaluation of potential carcinogenicity provides a possible means for distinguishing between genotoxic and non-genotoxic processes): As described in Section II, DINP is not genotoxic as evidenced by negative results in a number of short term tests including Salmonella, mouse lymphoma and micronucleus tests (Barber *et al.*, 2000; McKee *et al.*, 2000; Zeiger *et al.*, 1985).
- (d) Animal bioassay data in other sex-species combinations: As described above, DINP produces tubule cell carcinomas in male rats but not in female rats or in mice of either sex. This is consistent with the expected pattern of response for an alpha_{2u}-globulin mechanism. It also provides indirect evidence that, if there are other toxic processes associated with DINP treatment, they do not contribute to kidney cancer as no kidney tumors were found except in male rats and under conditions in which alpha_{2u}-globulin was increased.

EPA's guidance summarizes the evaluation of the three "must have" factors, plus additional evidence, as follows:

Confidence in determining which of the three categories [*i.e.*, compounds producing renal tumors in male rats attributable solely to chemically induced alpha_{2u}-globulin accumulation; compounds producing renal tubule tumors that are not linked to alpha_{2u}-globulin accumulation; compounds producing some renal tubule tumors in male rats attributable to the alpha_{2u}-globulin process and some attributable to other carcinogenic processes] applies depends on the comprehensiveness and consistency of the available data. If all the data (two species, two sex combination bioassay, all

⁸ Data for all categories of additional information listed by EPA are not required. As EPA states: "the information may not always be available; nor should this list be considered exhaustive." (EPA, 1991, p. 87).

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elements in XVII-A [the 3 specific findings described above], and additional information such as that described in XVII-B [including points a-d above]) are consistent with a role for chemically induced [alpha_{2u}-globulin], there is a high degree of confidence that the [alpha_{2u}-globulin] syndrome alone accounts for the renal tubule tumors. (EPA. 1991, p.88)

Application of this reasoning to the DINP data shows a high degree of confidence that the alpha_{2u}-globulin syndrome alone accounts for the renal tubule tumors observed in male rats treated with DINP. As documented above, there is a two-species, two-sex bioassay that provides data consistent with the alpha_{2u}-globulin process, *i.e.*, malignant tubule cell tumors in kidneys of male rats but not female rats or mice (Moore, 1998a and b). The three required criteria (Section XVII-A) are met: there is evidence of hyaline droplet accumulation, a demonstration that the accumulating protein in the hyaline droplets is alpha_{2u}-globulin, and histopathological evidence consistent with an alpha_{2u}-globulin process. There is also additional information as described in section XVII-B that is consistent with a role for chemically-induced alpha_{2u}-globulin. No data for DINP are inconsistent with an alpha_{2u}-globulin process. Thus, under EPA's guidance, an alpha_{2u}-globulin mediated process is the most plausible mechanism for kidney tumor induction, the male rat kidney tumors should be attributed to an alpha_{2u}-globulin process, and neither those tumors nor any associated renal toxicity should be used for human health hazard identification.

2. The DINP Data Meet the IARC Criteria for an Alpha_{2u}-Globulin Mechanism

A review of the significance of alpha_{2u}-globulin induction to human health was conducted in 1997 by the International Agency for Research on Cancer (IARC) (Swenberg, and Lehman-McKeeman, 1998). An expert panel reviewed the evidence for alpha_{2u}-globulin as a mechanism for renal-cell neoplasms and concluded that this mechanism was operative only in male rats and had no clinical significance for humans. The panel further determined that kidney tumors in male rats which are the consequence of an alpha_{2u}-globulin-mediated process should not be used in an assessment of human carcinogenic hazard. Finally, the IARC panel defined a set of criteria, similar to those established by the EPA, which could be used to determine whether a substance acts via an alpha_{2u}-process (Swenberg, and Lehman-McKeeman, 1998).

The IARC criteria, and how the DINP compare to those criteria, are as follows:

- (a) Lack of genotoxic activity (agent and/or metabolite) based on an overall evaluation of in-vitro and in-vivo data. As described in Section II of these comments, DINP has been tested in a number of in vivo and intro tests for genotoxic activity and all have produced negative results (Barber *et al.*, 2000; McKee *et al.*, 2000; Zeiger *et al.*, 1985).
- (b) Male rat specificity for nephropathy and renal tumorigenicity. As shown in Table 4 (above), the renal tumors were in male rats; there were none in female rats or in mice of either sex. The male rat specificity for an alpha_{2u}-globulin nephropathy is documented in Caldwell *et al.* (1999). Thus the male rat specificity for both

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nephropathy and renal tumorigenicity has been documented.

- (c) Induction of the characteristic sequence of histopathological changes in shorter-term studies of which protein droplet accumulation is obligatory. As described above, protein droplet accumulation is documented in Caldwell *et al.* (1999) along with evidence that the protein which is being accumulated is alpha_{2u}-globulin. Other aspects of characteristic pathology – including tubular regeneration and tubular hypertrophy in male but not female rat kidney – are also documented in Caldwell *et al.* (1999). Evidence of mineralization of renal tubules is documented in Moore (1998a).
- (d) Identification of the protein accumulating in tubular cells as alpha_{2u}-globulin. This was documented by Caldwell *et al.* (1999) and confirmed by Schoonhoven *et al.* (2001).
- (e) Reversible binding of the chemical or metabolite to alpha_{2u}-globulin. This is documented in Schoonhoven *et al.* (2001). *See also* Attachment E.
- (f) Induction of sustained increased cell proliferation in the renal cortex. This was documented in Caldwell *et al.* (1999) and confirmed by Schoonhoven *et al.* (2001) by a different technique.
- (g) Similarities in dose-response relationship of the tumor outcome with the histopathological end-points (protein droplets, alpha_{2u}-globulin accumulation, cell proliferation). Kidney tumors were found only after dietary administration of DINP at a level of 1.2% (733 mg/kg/day in the male rats). As documented in Caldwell *et al.* (1999), protein droplets and alpha_{2u}-globulin accumulation were significantly elevated in comparison to control values at 0.6% (307 mg/kg/day) but not at lower levels (307 mg/kg/day was the highest dose used in the Caldwell *et al.* study). As shown by Caldwell *et al.* (1999), cell proliferation was elevated at 0.6% in the diet, but was not significantly different from controls. Schoonhoven *et al.* (2001) reported a doubling in cell proliferation in animals given 900 mg/kg. Thus it is evident that significant effects in the critical parameters are found at doses approximating the tumorigenic levels.

Thus, DINP meets all of the IARC criteria, showing that the male rat kidney tumors associated with DINP treatment are the result of an alpha_{2u}-globulin-mediated process and are not relevant to humans.

**3. Expert Body Reviews Have Concluded that DINP Data Meet
the Criteria for an Alpha_{2u}-Globulin Mechanism**

Reviewing bodies have agreed the DINP data meet the criteria for an alpha_{2u}-globulin-mediated process and have therefore found that kidney tumors seen in male rats treated with DINP are not relevant for human risk assessment.

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The CPSC CHAP report states:

Male rat specificity in tumor response, lack of genotoxicity, histopathology findings of cytotoxicity and regeneration, α 2 μ -globulin accumulation, and demonstrated cell proliferation strongly support the criteria for demonstrating α 2 μ -globulin mechanism (IARC, 1998). Therefore, the renal tumors in male rats at the high dose of DINP are assumed to be rat specific and are not used to predict human cancer risk. (CPSC, 2001, p. 91)

The EU risk assessment states: “Pertaining to kidney tumours, the species and sex-specific alpha 2u globulin mechanism likely responsible for kidney tumours seen in male rats is not regarded as relevant to humans.” (ECB, 2003a, p. 223; ECB, 2003b, p. 14)

D. Exposure to DINP Does Not Result in Testicular Dysgenesis Syndrome

In the compilation of studies on DINP, under the heading “Mechanisms”, OEHHA lists “Testicular dysgenesis syndrome” (TDS) and references the paper published by Borch *et al.* (2004). However, based on all currently available data, DINP does not induce TDS and TDS should not be considered as a mechanism of toxicity for DINP.

The TDS term was first coined in 2001 when it was hypothesized that cases of abnormal spermatogenesis, cryptorchidism (undescended testicles), penile malformations such as hypospadias, and incidences of testicular cancer observed in humans may all have a common etiology (Skakkebaek *et al.*, 2001). The hypothesis states that these clinical problems may result from an irreversible developmental disorder early in fetal life consequential to either a genetic predisposition and/or environmental insult(s). Currently, no biological mechanism is defined for TDS, but it is theorized that abnormal spermatogenesis and testicular cancer may be the result of disturbed Sertoli cell function while hypospadias and cryptorchidism may result from decreased Leydig cell function (Wohlfahrt-Veje *et al.*, 2009).

There have been several rigorous scientific reviews of DINP, including those of the National Toxicology Program (NTP) Center for the Evaluation of Risks to Human Reproduction (CERHR) (NTP CERHR, 2000), the Consumer Product Safety Commission (CPSC) Chronic Hazard Advisory Panel (CHAP) for DINP (CPSC, 2001), and the European Union Risk Assessment for DINP (ECB 2003a;b). In all of the summaries of reproductive, developmental, and chronic carcinogenicity studies, DINP has not been identified as producing any adverse TDS-like effects.

In chronic 2-year carcinogenicity reports published by Moore *et al.* (1998a) (daily exposure to 0, 500, 1500, 6000, or 12000 ppm) and Lington *et al.* (1997) (daily exposure to 0, 300, 3000, or 6000 ppm), benign testicular interstitial cell tumors were found in nearly all control and F344 rats treated chronically with DINP. However, this finding is not considered relevant since the incidences of the tumors were found to be within the historical control range and F344 rats normally display a high incidence of testicular tumors. Similarly, a 2-year carcinogenicity study involving Sprague Dawley rats exposed daily to 0, 500, 5000, or 10000 ppm to a DINP

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mixture that was never commercially produced, both testicular (interstitial) cell hyperplasia and tumors were elevated above concurrent controls in the only exposure group examined, the high dose exposure group (Biodynamics, 1986). The incidence of the testicular tumors in the high dose animals were significantly above those in the controls, but within the range of historical control values and are therefore not considered reliable for interpretation.

In a one-generation reproductive and developmental toxicity study (Exxon Biomedical Sciences, 1996), rats were administered 0.5, 1, or 1.5% DINP from 10 weeks prior to mating, through gestation, and ending on post natal day (PND) 21. Pertaining to P1 male organ toxicity, there was a statistically significant increase in the mean absolute and relative right testis weight, left testis and right epididymis weights and the mean relative left epididymis and seminal vesicle weights in the high-dose males compared with controls. It was not determined if any structural changes occurred in reproductive organs at any dose level; microscopic evaluation was not performed on any organs in both sexes. Thus significance of organ weight changes could not be assessed because of the limitation of the study. However, there were no statistically significant differences in male mating, male fertility, female fertility, female fecundity, or female gestational indices between treated and control animals.

In a two generation study (Waterman *et al.*, 2000), P1 males and females received test material daily for at least ten weeks prior to mating and during the mating period. Additionally, P1 female animals received test material during the gestation and postpartum periods, until weaning of the F1 offspring on Post Partum Day (PPD) 21. P2(F1) males were dosed from PND 21 for at least 10 weeks prior to mating and through the mating period for F2 litters, until sacrificed following delivery of their last litter sired. P2(F1) females were dosed from PND 21 for at least 10 weeks prior to mating, during mating, gestation, lactation, and until they were sacrificed following weaning of the F2 animals on PPD 21. There were no statistically significant differences in male mating, male fertility, female fertility, female fecundity or female gestational indices in P1 generation. A slight decrease, not statistically significant, of male mating, male fertility, female fertility, and female fecundity indices was observed in P2 generation. Mean days of gestation of the P1/P2 treated and control animals were essentially equivalent. There were no adverse testicular effects reported for either the P1 or P2 generation.

Sharpe (2003) proposed that suppression of fetal androgen production and/or increased estrogen exposure might underlie the occurrence of TDS with respect to certain phthalates. However, the data for DINP are inconsistent with respect to anti-androgenic effects in young male rats. Two studies, which used an unrealistically high doses of DINP administered by gavage, resulted in a questionably significant increase in malformation of the male reproductive tract (Gray *et al.*, 2000) or decreased testosterone in male rats (Borch *et al.*, 2004). In contrast, no anti-androgenic effects were observed in male offspring of pregnant rats exposed to higher levels of DINP in the diet (Masutomi, *et al.*, 2003).

The study conducted by Gray *et al.* (2000) shows a low incidence of effects without any dose response and with effects of unclear significance. As infants, male rats were exposed to a single 750 mg/kg dose of DINP between gestation day 14 and post natal day 3. The authors reported that some males displayed retained areolas (22% reported as statistically significant). No other single endpoint (nipple retention, epididymal agenesis, fluid filled testes, and testes

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weight) on its own was significantly different to control values. However, the authors pooled all observed effects to produce the 7.7% adverse incidence reported in the study. Only by pooling different effects was statistical significance demonstrated. This type of data manipulation is not routinely performed in toxicological safety evaluations, nor is it considered good statistical practice. It should also be noted that Gray *et al.* (2000) did not see any effects on anogenital distance or on reduction of testosterone levels in the blood with DINP treated animals. Based on the above points it is unclear whether adverse effects have been found for DINP in this study.

Likewise, the paper by Borch *et al.* (2004) does not present data demonstrating that DINP induces TDS and should not be considered as evidence for a mechanism of toxicity. In this report, 32 pregnant female rats were exposed to either 300 mg/kg-bw DEHP or 750 mg/kg-bw DINP, alone or in combination, from gestation day 7 to gestation day 21. The dams were sacrificed on gestation day 21 and the pups were harvested for analysis of testicular testosterone production, testicular testosterone content, plasma testosterone levels, and plasma luteinizing hormone (LH) levels. The results indicate that testicular testosterone production and testicular testosterone content were significantly decreased in the DINP exposed pups while plasma testosterone and plasma LH levels were unaltered. However, no mechanism of toxicity can be determined from this paper since it is limited by several confounding factors. First, the dose was administered via a single oral gavage exposure each days of testing. This method of administration can result in the overwhelming of normal detoxifying processes which can lead to overt toxicity. Second, there were no adverse phenotypic effects reported in the study. Therefore it is unclear if the decrease in testosterone content is in fact a toxicologically significant response. Third, while DEHP and DINP alone appeared to induce a decrease in testosterone content, there was no indication of a modulating effect of DINP on DEHP when co-administered. Finally, the authors sampled testosterone levels on gestation day 21, a time point after the developmental surge of testosterone that occurs during gestation day 16-18 in the rat. After gestation day 18, plasma testosterone levels are naturally declining in the fetal rat.

In conclusion, there is no evidence that DINP induces TDS in laboratory animals. As stated by the CERHR expert panel (NTP CERHR, 2000):

Reproductive performance and histological effects on gonads and accessory sex organs were assessed in one- and two-generation dietary studies. Parental doses of up to 0.8% in feed (665–779 [M] and 696–802[F] mg/kg bw/day) did not affect fertility or sex organ histology in either the F0 or F1 male or female pups. A 13-week gavage study in adult marmosets resulted in no evidence of microscopic testicular changes at doses that did adversely affect body weight gain (2,500 mg/kg bw/day). Testicular lesions were not observed in prepubertal cynomolgus monkeys that were gavaged for 2 weeks with 500 mg/kg bw/day, reportedly the maximum dose that can be absorbed by the monkeys. Chronic 2-year studies in rats and mice gave no gross or histologic evidence of effects on testes or ovaries at doses that did cause liver and kidney effects and other clinical signs of toxicity. Thus, the data are sufficient to conclude that neither the reproductive organs nor fertility are affected by extended oral exposure to DINP.

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CONCLUSION

The foregoing information is presented to assist the CIC in developing a recommendation concerning the level of priority that should be assigned to DINP for possible evaluation for listing under Proposition 65. For the reasons presented, ExxonMobil believes the data support the conclusion that the cancer findings in rodent bioassays of DINP are not relevant to humans. Therefore, ExxonMobil respectfully submits that DINP should not be listed under Proposition 65 as a carcinogen. The ranking of DINP for development of hazard identification materials should be “no priority” or, at most, “low priority.”

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NOTE: ExxonMobil would be pleased upon request to provide a copy of any of these studies.

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COMMENTS ON PRIORITIZATION OF DIISONONYL PHTHALATE (DINP)
Prioritization: Chemicals For Consulation By The Carcinogen Identification Committee

Attachments

The following attachments are provided as separate pdfs.

- A Statement of Ruth Angela Roberts, Ph.D.
- B Statement of James Klaunig, Ph.D.
- C Statement of Gary Williams, M.D. and Michael Iatropoulos, M.D., Ph.D.
- D Statement of Richard Irons, Ph.D.
- E Statement of James Swenberg, Ph.D.

Also provided as a separate pdf is the full text of Klaunig *et al.*, 2003.